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=> fil medline

FILE 'MEDLINE' ENTERED AT 07:52:38 ON 13 NOV 2000

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=> d his


     (FILE 'MEDLINE' ENTERED AT 07:40:06 ON 13 NOV 2000)
               DEL HIS Y

     FILE 'MEDLINE' ENTERED AT 07:51:19 ON 13 NOV 2000
L1            124 S GUANOSINE DIPHOSPHATE FUCOSE/CT
L2             39 S L1/MAJ
L3             16 S L2 (L) (CS OR BI OR IP)/CT

     FILE 'MEDLINE' ENTERED AT 07:52:38 ON 13 NOV 2000

=> d .med 1-16


L3   ANSWER 1 OF 16  MEDLINE
AN   2000223138     MEDLINE
DN   20223138
TI   A bifunctional epimerase-reductase acts downstream of the MUR1 gene
     product and completes the de novo synthesis of GDP-L-fucose in
     Arabidopsis.
AU   Bonin C P; Reiter W D
CS   University of Connecticut, Department of Molecular and Cell Biology,
     Storrs, CT 06269, USA.
SO   PLANT JOURNAL, (2000 Mar) 21 (5) 445-54.
     Journal code: BRU. ISSN: 0960-7412.
CY   ENGLAND: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200007
EW   20000704
AB   L-Fucose is a monosaccharide found as a component of glycoproteins and
     cell wall polysaccharides in higher plants. The MUR1 gene of Arabidopsis
     thaliana encodes a GDP-D-mannose 4,6-dehydratase catalyzing the first
step
     in the de novo synthesis of GDP-L-fucose from GDP-D-mannose (Bonin et al.
     1997, Proc. Natl Acad. Sci. USA, 94, 2085-2090). Plant genes encoding the
```

subsequent steps in L-fucose synthesis (3,5-epimerization and 4-reduction)
have not been described previously. Based on sequence similarities to a bacterial gene involved in capsule synthesis we have cloned a gene from Arabidopsis, now designated GER1, which encodes a bifunctional 3, 5-epimerase-4-reductase in L-fucose synthesis. The combined action of the MUR1 and GER1 gene products converts GDP-D-mannose to GDP-L-fucose in vitro demonstrating that this entire nucleotide-sugar interconversion pathway could be reconstituted using plant genes expressed in Escherichia coli. In vitro assays indicated that the GER1 protein does not act as a GDP-D-mannose 3, 5-epimerase, an enzymatic activity involved in the de novo synthesis of GDP-L-galactose and L-ascorbic acid. Similarly, L-ascorbate levels in GER1 antisense plants were unchanged indicating that
GDP-D-mannose 3,5-epimerase is encoded by a separate gene.

CT Check Tags: Human; Support, U.S. Gov't, Non-P.H.S.
Amino Acid Sequence
*Arabidopsis: EN, enzymology
Arabidopsis: ME, metabolism
Base Sequence
Carbohydrate Epimerases: GE, genetics
*Carbohydrate Epimerases: ME, metabolism
DNA Primers
Escherichia coli: GE, genetics
*Genes, Plant
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Introns
Molecular Sequence Data
Oligonucleotides, Antisense: GE, genetics
*Oxidoreductases: ME, metabolism
Plants, Transgenic
Sequence Homology, Amino Acid
*Sugar Alcohol Dehydrogenases: ME, metabolism

L3 ANSWER 2 OF 16 MEDLINE
AN 1999410404 MEDLINE
DN 99410404
TI Stereochemical course and steady state mechanism of the reaction catalyzed by the GDP-fucose synthetase from Escherichia coli.
AU Menon S; Stahl M; Kumar R; Xu G Y; Sullivan F
CS Wyeth Research, Cambridge, Massachusetts 02140, USA.. smenon@genetics.com
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Sep 17) 274 (38) 26743-50.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199912
EW 19991203
AB Recently the genes encoding the human and Escherichia coli GDP-mannose dehydratase and GDP-fucose synthetase (GFS) protein have been cloned and it has been shown that these two proteins alone are sufficient to convert GDP mannose to GDP fucose in vitro. GDP-fucose synthetase from E. coli is a novel dual function enzyme in that it catalyzes epimerizations and a reduction reaction at the same active site. This aspect separates fucose biosynthesis from that of other deoxy and dideoxy sugars in which the

epimerase and reductase activities are present on separate enzymes encoded
by separate genes. By NMR spectroscopy we have shown that GFS catalyzes the stereospecific hydride transfer of the ProS hydrogen from NADPH to carbon 4 of the mannose sugar. This is consistent with the stereospecificity observed for other members of the short chain dehydrogenase reductase family of enzymes of which GFS is a member. Additionally the enzyme is able to catalyze the epimerization reaction in the absence of NADP or NADPH. The kinetic mechanism of GFS as determined by product inhibition and fluorescence binding studies is consistent with a random mechanism. The dissociation constants determined from fluorescence studies indicate that the enzyme displays a 40-fold stronger affinity for the substrate NADPH as compared with the product NADP and utilizes NADPH preferentially as compared with NADH. This study on GFS, a unique member of the short chain dehydrogenase reductase family, coupled with that of its recently published crystal structure should aid in the development of antimicrobial or anti-inflammatory compounds that act by blocking selectin-mediated cell adhesion.

CT *Carbohydrate Epimerases: ME, metabolism
Catalysis
Chromatography, Paper
*Escherichia coli: EN, enzymology
Guanosine Diphosphate: ME, metabolism
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Hydrogen-Ion Concentration
*Ketone Oxidoreductases: ME, metabolism
Models, Chemical
*Multienzyme Complexes: ME, metabolism
Nuclear Magnetic Resonance
NADP: ME, metabolism
Spectrometry, Fluorescence
Stereoisomerism

L3 ANSWER 3 OF 16 MEDLINE
AN 1999354247 MEDLINE
DN 99354247
TI Activity of enzymes catalyzing formation of beta-L-fucosyl phosphate and GDP-beta-L-fucose in amphibian tissues and their application in chemo-enzymic synthesis of GDP-beta-L-fucose.
AU Druzhinina T N; Utkina N S; Chan K; Strecker G; Shibaev V N
CS Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, 117913, Russia.
SO BIOCHEMISTRY, (1999 Jul) 64 (7) 783-7.
Journal code: CSQ. ISSN: 0006-2979.
CY RUSSIA: Russian Federation
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199911
EW 19991101
AB Amphibian oviduct and liver were shown to contain enzymes that catalyze the formation of GDP-beta-L-fucose from GDP-alpha-D-mannose or L-fucose. The conversion of L-fucose into beta-L-fucopyranosyl phosphate was achieved on a preparative scale using high-activity fucokinase in toad liver extracts. For chemo-enzymic preparation of GDP-beta-L-fucose, a convenient modification for pyrophosphate synthesis through phosphomorpholidate which does not require anhydrous conditions is

suggested.
CT Check Tags: Animal; Female; Support, Non-U.S. Gov't
Bufo bufo
Catalysis
*Enzymes: ME, metabolism
*Fucose: AA, analogs & derivatives
Fucose: BI, biosynthesis
***Guanosine Diphosphate Fucose: BI, biosynthesis**
*Hexosephosphates: BI, biosynthesis
*Liver: EN, enzymology
*Oviducts: EN, enzymology
Pleurodeles

L3 ANSWER 4 OF 16 MEDLINE
AN 1999164952 MEDLINE
DN 99164952
TI Mutation in GDP-fucose synthesis genes of Sinorhizobium fredii alters Nod factors and significantly decreases competitiveness to nodulate soybeans.
AU Lamrabet Y; Bellogin R A; Cubo T; Espuny R; Gil A; Krishnan H B; Megias M;
Ollero F J; Pueppke S G; Ruiz-Sainz J E; Spaink H P; Tejero-Mateo P; Thomas-Oates J; Vinardell J M
CS Departamento de Microbiologia y Parasitologia, Facultad de Farmacia, Universidad de Sevilla, Spain.
SO MOLECULAR PLANT-MICROBE INTERACTIONS, (1999 Mar) 12 (3) 207-17.
Journal code: A9P. ISSN: 0894-0282.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF072888
EM 199905
EW 19990504
AB We mutagenized Sinorhizobium fredii HH103-1 with Tn5-B20 and screened about 2,000 colonies for increased beta-galactosidase activity in the presence of the flavonoid naringenin. One mutant, designated SVQ287, produces lipochitooligosaccharide Nod factors (LCOs) that differ from those of the parental strain. The nonreducing N-acetylglucosamine residues of all of the LCOs of mutant SVQ287 lack fucose and 2-O-methylfucose substituents. In addition, SVQ287 synthesizes an LCO with an unusually long, C20:1 fatty acyl side chain. The transposon insertion of mutant SVQ287 lies within a 1.1-kb HindIII fragment. This and an adjacent 2.4-kb HindIII fragment were sequenced. The sequence contains the 3' end of noeK, nodZ, and noeL (the gene interrupted by Tn5-B20), and the 5' end of nolK, all in the same orientation. Although each of these genes has a similarly oriented counterpart on the symbiosis plasmid of the broad-host-range Rhizobium sp. strain NGR234, there are significant differences in the noeK/nodZ intergenic region. Based on amino acid sequence homology, noeL encodes GDP-D-mannose dehydratase, an enzyme involved in the synthesis of GDP-L-fucose, and nolK encodes a NAD-dependent nucleotide sugar epimerase/dehydrogenase. We show that expression of the noeL gene is under the control of NodD1 in S. fredii and is most probably mediated by the nod

box that precedes nodZ. Transposon insertion into neoL has two impacts on symbiosis with Williams soybean: nodulation rate is reduced slightly and competitiveness for nodulation is decreased significantly. Mutant SVQ287 retains its ability to form nitrogen-fixing nodules on other legumes, but final nodule number is attenuated on Cajanus cajan.

CT Check Tags: Support, Non-U.S. Gov't
beta-Galactosidase: ME, metabolism
Amino Acid Sequence
Base Sequence
Carbohydrate Sequence
DNA, Bacterial
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Molecular Sequence Data
*Mutation
*Nitrogen Fixation: GE, genetics
Open Reading Frames
Phenotype
*Plant Proteins: GE, genetics
Rhizobium: EN, enzymology
*Rhizobium: GE, genetics
Sequence Homology, Amino Acid
*Soybeans: PH, physiology

L3 ANSWER 5 OF 16 MEDLINE
AN 1999119297 MEDLINE
DN 99119297
TI A high-yield, enzymatic synthesis of GDP-D-[3H]arabinose and GDP-L-[3H]fucose.
AU Mengeling B J; Turco S J
CS Department of Biochemistry, University of Kentucky Medical Center, 800 Rose Street, Lexington, Kentucky, 40536-0298, USA.
NC AI 20941 (NIAID)
SO ANALYTICAL BIOCHEMISTRY, (1999 Feb 1) 267 (1) 227-33.
Journal code: 4NK. ISSN: 0003-2697.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
EW 19990504
AB For assays involving glycosyltransferases or transporters, several GDP-sugars are either commercially unavailable or expensive. We describe an enzymatic synthesis of GDP-d-[3H]arabinosep and GDP-l-[3H]fucose that yields 66-95% nucleotide-sugar from the appropriate radiolabeled sugar in less than 30 min. The coupled reaction requires Mg2+, ATP, and GTP along with the appropriate radioactive monosaccharide, sugar-1-kinase, and pyrophosphorylase. The latter two activities are present in a cytosolic fraction of Crithidia fasciculata, which is easily grown at room temperature in simple culture medium without serum or added CO2. Addition of commercial yeast inorganic pyrophosphatase shifts the equilibrium of the pyrophosphorylase reaction toward nucleotide-sugar formation. To verify that these nucleotide-sugars are biologically active, we tested their ability to serve as substrates for glycosyltransferases. GDP-l-[3H]fucose functions as the donor substrate for recombinant human fucosyltransferase V, and GDP-d-[3H]arabinosep serves as the donor substrate for the arabinosyltransferase activities present in Leishmania major microsomes. Copyright 1999 Academic Press.

LewisX, and various fucosylated blood group antigens. To date, the molecular anomaly in these patients has not been identified. We localized the defect in LAD II to the de novo pathway of GDP-fucose biosynthesis, by inducing cell-surface expression of fucosylated glycoconjugates after exposure of lymphoblastoid cell lines from the LAD II patients to exogenous fucose. This defect is not restricted to hematopoietic cells, since similar findings were elicited in both human umbilical vein endothelial cells (HUVEC) and fibroblasts derived from an affected abortus. We have used these LAD II endothelial cells to examine the consequence of fucosylation of endothelial cells on the rolling of normal neutrophils in an in vitro assay. Neutrophil rolling on LPS-treated normal and LAD II HUVEC was inhibited by an E-selectin monoclonal antibody at both high and low shear rates. LAD II HUVEC lacking fucosylated glycoproteins supported leukocyte rolling to a similar degree as normal HUVEC or LAD II cells that were fucose-fed. At low shear rates, an L-selectin antibody inhibited neutrophil rolling to a similar degree whether the LAD II cells had been fucose-fed or not. These findings suggest that fucosylation of nonlymphoid endothelial cells does not play a major role in neutrophil rolling and that fucose is not a critical moiety on the L-selectin ligand(s) on endothelial cells of the systemic vasculature.

CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Adult
Cell Line
Cell Movement
*Endothelium, Vascular: ME, metabolism
Glycoconjugates: ME, metabolism
***Guanosine Diphosphate Fucose: BI, biosynthesis**
L-Selectin: PH, physiology
*Leukocyte-Adhesion Deficiency Syndrome: ME, metabolism
*Neutrophils: PH, physiology

L3 ANSWER 8 OF 16 MEDLINE
AN 1998192611 MEDLINE
DN 98192611
TI Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis in vitro.
AU Sullivan F X; Kumar R; Kriz R; Stahl M; Xu G Y; Rouse J; Chang X J; Boodhoo A; Potvin B; Cumming D A
CS Small Molecule Drug Discovery, Genetics Institute, Inc., 424 Wilkinway, Edmonton, Alberta T6M 2H8, Canada.. fsullivan@genetics.com
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Apr 3) 273 (14) 8193-202. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-AF042377
EM 199807
EW 19980702
AB We have cloned the cDNA encoding human GDP-mannose 4,6-dehydratase, the first enzyme in the pathway converting GDP-mannose to GDP-fucose. The message is expressed in all tissues and cell lines examined, and the cDNA complements Lec13, a Chinese Hamster Ovary cell line deficient in

GDP-mannose 4,6-dehydratase activity. The human GDP-mannose 4,6-dehydratase polypeptide shares 61% identity with the enzyme from Escherichia coli, suggesting broad evolutionary conservation. Purified recombinant enzyme utilizes NADP+ as a cofactor and, like its E. coli counterpart, is inhibited by GDP-fucose, suggesting that this aspect of regulation is also conserved. We have isolated the product of the dehydratase reaction, GDP-4-keto-6-deoxymannose, and confirmed its structure by electrospray ionization-mass spectrometry and high field NMR.

Using purified recombinant human GDP-mannose 4,6-dehydratase and FX protein (GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase), we show that the two proteins alone are sufficient to convert GDP-mannose to GDP-fucose in vitro. This unequivocally demonstrates that the epimerase and reductase activities are on a single polypeptide. Finally, we show that the two homologous enzymes from E. coli are sufficient to carry out the same enzymatic pathway in bacteria.

CT Check Tags: Animal; Human
Amino Acid Sequence
Base Sequence
Cloning, Molecular
DNA, Complementary: GE, genetics
DNA, Complementary: IP, isolation & purification
Escherichia coli
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Guanosine Diphosphate Fucose: GE, genetics
Hamsters
*Hydro-Lyases: GE, genetics
Hydro-Lyases: ME, metabolism
Molecular Sequence Data
Sequence Alignment
Transfection

L3 ANSWER 9 OF 16 MEDLINE
AN 1998132401 MEDLINE
DN 98132401
TI Identification of the fucose synthetase gene in the colanic acid gene cluster of Escherichia coli K-12.
AU Andrianopoulos K; Wang L; Reeves P R
CS Department of Microbiology, The University of Sydney, New South Wales, Australia.
SO JOURNAL OF BACTERIOLOGY, (1998 Feb) 180 (4) 998-1001.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199805
EW 19980502
AB GDP-L-fucose, the substrate for fucosyltransferases for addition of fucose to polysaccharides or glycoproteins in both procaryotes and eucaryotes, is made from GDP-D-mannose. L-Fucose is a component of bacterial surface antigens, including the extracellular polysaccharide colanic acid produced

by most Escherichia coli strains. We previously sequenced the E. coli colanic acid gene cluster and identified one of the GDP-L-fucose biosynthetic pathway genes, gmd. We report here the identification of the gene (fcl), located downstream of gmd, encoding the fucose synthetase.

CT Check Tags: Comparative Study; Support, Non-U.S. Gov't
Amino Acid Sequence
*Carbohydrate Epimerases: GE, genetics
Carrier Proteins: GE, genetics
Escherichia coli: EN, enzymology
*Escherichia coli: GE, genetics
*Genes, Bacterial
***Guanosine Diphosphate Fucose: BI, biosynthesis**
*Ketone Oxidoreductases: GE, genetics
Molecular Sequence Data
*Multienzyme Complexes: GE, genetics
Multigene Family
*Polysaccharides: BI, biosynthesis
Polysaccharides: GE, genetics
*Polysaccharides, Bacterial: BI, biosynthesis
Polysaccharides, Bacterial: GE, genetics
Sequence Homology, Amino Acid

L3 ANSWER 10 OF 16 MEDLINE
AN 97345676 MEDLINE
DN 97345676
TI The nodulation gene nolK of Azorhizobium caulinodans is involved in the formation of GDP-fucose from GDP-mannose.
AU Mergaert P; Van Montagu M; Holsters M
CS Department of Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Universiteit Gent, Belgium.
SO FEBS LETTERS, (1997 Jun 9) 409 (2) 312-6.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199709
EW 19970903
AB The nolK gene of Azorhizobium caulinodans is essential for the incorporation of a fucosyl group in Nod factors. A NAD(P)-binding site is present in the NolK amino acid sequence and the gene is homologous to Escherichia coli genes, presumably involved in GDP-fucose synthesis. Protein extracts of A. caulinodans, overexpressing nolK, have an enzyme activity that synthesizes GDP-fucose from GDP-mannose. nolK most probably encodes a 4-reductase performing the last step in GDP-fucose synthesis. Wild-type A. caulinodans produces a population of fucosylated and non-fucosylated molecules but the nolK-overexpressing strain produces only fucosylated Nod factors. Thus, the production of activated fucosyl donors is a rate-limiting step in Nod factor fucosylation.

CT Check Tags: Support, Non-U.S. Gov't
Fucose: ME, metabolism
Fucosyltransferases: ME, metabolism
*Genes, Nitrogen Fixation: PH, physiology
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Guanosine Diphosphate Fucose: CH, chemistry
*Guanosine Diphosphate Mannose: BI, biosynthesis

Guanosine Diphosphate Mannose: CH, chemistry
*Rhizobiaceae: GE, genetics
Rhizobiaceae: PH, physiology

L3 ANSWER 11 OF 16 MEDLINE
AN 97203191 MEDLINE
DN 97203191
TI The MUR1 gene of Arabidopsis thaliana encodes an isoform of GDP-D-mannose-4,6-dehydratase, catalyzing the first step in the de novo synthesis of GDP-L-fucose.
AU Bonin C P; Potter I; Vanzin G F; Reiter W D
CS Department of Molecular and Cell Biology, University of Connecticut, Storrs 06269, USA.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Mar 4) 94 (5) 2085-90.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U81805
EM 199706
AB GDP-L-fucose is the activated nucleotide sugar form of L-fucose, which is a constituent of many structural polysaccharides and glycoproteins in various organisms. The de novo synthesis of GDP-L-fucose from GDP-D-mannose encompasses three catalytic steps, a 4,6-dehydration, a 3,5-epimerization, and a 4-reduction. The mur1 mutant of Arabidopsis is deficient in L-fucose in the shoot and is rescued by growth in the presence of exogenously supplied L-fucose. Biochemical assays of the de novo pathway for the synthesis of GDP-L-fucose indicated that mur1 was blocked in the first nucleotide sugar interconversion step, a GDP-D-mannose-4,6-dehydratase. An expressed sequence tag was identified that showed significant sequence similarity to proposed bacterial GDP-D-mannose-4,6-dehydratases and was tightly linked to the mur1 locus. A full-length clone was isolated from a cDNA library, and its coding region was expressed in Escherichia coli. The recombinant protein exhibited GDP-D-mannose-4,6-dehydratase activity in vitro and was able to complement mur1 extracts in vitro to complete the pathway for the synthesis of GDP-L-fucose. All seven mur1 alleles investigated showed single point mutations in the coding region for the 4,6-dehydratase, confirming that it represents the MUR1 gene.
CT Check Tags: Support, U.S. Gov't, Non-P.H.S.
Amino Acid Sequence
Arabidopsis: EN, enzymology
*Arabidopsis: GE, genetics
Base Sequence
Blotting, Southern
Carbohydrate Epimerases: ME, metabolism
Chromatography, Thin Layer
Cloning, Molecular
DNA, Complementary: GE, genetics
Escherichia coli: GE, genetics
Gene Expression: GE, genetics
*Genes, Plant

***Guanosine Diphosphate Fucose: BI, biosynthesis**
Hydro-Lyases: CH, chemistry
*Hydro-Lyases: GE, genetics
Hydro-Lyases: ME, metabolism
Isoenzymes: CH, chemistry
Isoenzymes: GE, genetics
Isoenzymes: ME, metabolism
Molecular Sequence Data
Molecular Structure
Mutation: GE, genetics
Sequence Alignment
Sugar Alcohol Dehydrogenases: ME, metabolism

L3 ANSWER 12 OF 16 MEDLINE
AN 93265491 MEDLINE
DN 93265491
TI Large-scale synthesis of beta-L-fucopyranosyl phosphate and the preparation of GDP-beta-L-fucose.
AU Adelhorst K; Whitesides G M
CS Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138..
NC GM 30367 (NIGMS)
SO CARBOHYDRATE RESEARCH, (1993 Apr 7) 242 69-76.
Journal code: CNY. ISSN: 0008-6215.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199308
AB A practical 15-mmol large-scale synthesis of beta-L-fucopyranosyl dicyclohexylammonium phosphate from L-fucose in 63% overall yield was developed. The synthesis took advantage of a neighboring Bz-2 group participating in a Koenigs-Knorr-like glycosylation. The sugar phosphate was transformed into the activated sugar nucleoside, guanosine diphosphate beta-L-fucopyranose, on a gram scale.
CT Check Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Carbohydrate Conformation
*Fucose: AA, analogs & derivatives
Fucose: CH, chemistry
Fucose: CS, chemical synthesis
Guanosine Diphosphate Fucose: CH, chemistry
***Guanosine Diphosphate Fucose: CS, chemical synthesis**
Hexosephosphates: CH, chemistry
*Hexosephosphates: CS, chemical synthesis
Indicators and Reagents
Nuclear Magnetic Resonance

L3 ANSWER 13 OF 16 MEDLINE
AN 86322014 MEDLINE
DN 86322014
TI Two Chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose.
AU Ripka J; Adamany A; Stanley P
NC R01 CA36434 (NCI)
CA90173 (NCI)

3PO CA13330 (NCI)
SO ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1986 Sep) 249 (2) 533-45. Journal code: 6SK. ISSN: 0003-9861.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 198612
AB A biochemical basis for the pea and lentil lectin resistance of two Chinese hamster ovary (CHO) cell mutants, Lec13 and Lec13A, was investigated. Studies of the G glycopeptides of vesicular stomatitis virus grown in the mutants indicated that Lec13 cells essentially lack the ability to add fucose to complex carbohydrates while Lec13A cells synthesize significant proportions of fucosylated, complex moieties. However, both mutants were known to be reverted to lectin sensitivity by growth in L-fucose, making them similar to the mouse lymphoma mutant, PLR1.3, which is defective in the conversion of GDP-mannose to GPD-fucose [M. L. Reitman, I. S. Trowbridge, and S. Kornfeld (1980) J. Biol. Chem. 255, 9900-9906]. Optimal conditions for the production of GDP-fucose from GDP-mannose by CHO cytosol were found to occur at pH 8 in the presence of 7.5 microM GDP-mannose, 15 mM Mg2+, 0.2 mM NAD+, 0.2 mM NADPH, 10 mM niacinamide, 5 mM ATP, and 50 mM Tris-HCl. Under these conditions, Lec13 cytosol produced no detectable GDP-fucose nor GDP-sugar intermediates while Lec13A cytosol produced significant quantities of both. Mixing experiments with Lec13 cytosol identified the first enzyme of the conversion pathway (GDP-mannose 4,6-dehydratase, EC 4.2.1.47) as the site of the block. In addition to being markedly reduced, the Lec13A 4,6-dehydratase activity was relatively insensitive to changes in pH in comparison to the activity in parental cytosol, suggesting that Lec13A cells might possess a structurally altered GDP-mannose 4,6-dehydratase enzyme.
CT Check Tags: Animal; Female; In Vitro; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Chromatography, Affinity
Cricetulus
***Guanosine Diphosphate Fucose: BI, biosynthesis**
*Guanosine Diphosphate Mannose: ME, metabolism
Hamsters
Hydro-Lyases: DF, deficiency
Hydro-Lyases: GE, genetics
Mutation
*Nucleoside Diphosphate Sugars: BI, biosynthesis
*Nucleoside Diphosphate Sugars: ME, metabolism
*Ovary: ME, metabolism

L3 ANSWER 14 OF 16 MEDLINE
AN 85027217 MEDLINE
DN 85027217
TI Study of the conversion of GDP-mannose into GDP-fucose in Nereids: a biochemical marker of oocyte maturation.
AU Bulet P; Hoflack B; Porchet M; Verbert A
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1984 Oct 15) 144 (2) 255-9. Journal code: EMZ. ISSN: 0014-2956.
CY GERMANY, WEST: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English

FS Priority Journals; Cancer Journals
EM 198502
AB Homogenates of Perinereis cultrifera oocytes were found to transform GDP-D-mannose into another sugar nucleotide. Ultraviolet absorption spectra, chromatographic behaviour, gas-liquid chromatography coupled to mass spectrometry analysis revealed that GDP-D-mannose had been converted into GDP-L-fucose. This conversion is a multi-step reaction as proved by the involvement of two intermediates identified as GDP-4-oxo-6-deoxy-D-mannose and GDP-4-oxo-6-deoxy-L-galactose, this latter being reduced by NADPH to give GDP-L-fucose. It is shown that the enzymatic activities responsible for the conversion of GDP-D-mannose into GDP-L-fucose is recovered only in oocytes and is not present in the other coelomic cells (i.e. coelomocytes). More interesting is the fact that maximum activity is recovered at a well defined stage of the hormone-controlled oogenesis. Thus, this enzymatic system appears as a biochemical marker of the oocyte maturation in P. cultrifera.
CT Check Tags: Animal; Female; Support, Non-U.S. Gov't
Biotransformation
Chromatography, Paper
***Guanosine Diphosphate Fucose: BI, biosynthesis**
*Guanosine Diphosphate Mannose: ME, metabolism
*Nucleoside Diphosphate Sugars: BI, biosynthesis
*Nucleoside Diphosphate Sugars: ME, metabolism
NAD: PH, physiology
NADP: PH, physiology
Oocytes: GD, growth & development
Oocytes: ME, metabolism
Oxidation-Reduction
*Polychaeta: ME, metabolism
Spectrum Analysis, Mass

L3 ANSWER 15 OF 16 MEDLINE
AN 82231342 MEDLINE
DN 82231342
TI Abrupt induction of GDP-fucose: asialo GM1 fucosyltransferase in the small intestine after conventionalization of germ-free mice.
AU Umesaki Y; Sakata T; Yajima T
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1982 Mar 30) 105 (2) 439-43.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198210
CT Check Tags: Animal; Comparative Study; Female
Enzyme Induction
*Germ-Free Life
***Guanosine Diphosphate Fucose: BI, biosynthesis**
*Intestine, Small: EN, enzymology
Intestine, Small: MI, microbiology
Mice
Mice, Inbred ICR
*Nucleoside Diphosphate Sugars: BI, biosynthesis

Time Factors

L3 ANSWER 16 OF 16 MEDLINE
AN 76134384 MEDLINE
DN 76134384
TI A simple and efficient method for the preparation of GDP-fucose.
AU Prohaska R; Schenkel-Brunner H
SO ANALYTICAL BIOCHEMISTRY, (1975 Dec) 69 (2) 536-44.
Journal code: 4NK. ISSN: 0003-2697.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197606
CT Check Tags: Animal
Adenosine Triphosphate: PD, pharmacology
***Guanosine Diphosphate Fucose: BI, biosynthesis**
Hydrogen-Ion Concentration
Kinetics
Magnesium: PD, pharmacology
*Nucleoside Diphosphate Sugars: BI, biosynthesis
Submandibular Gland: DE, drug effects
*Submandibular Gland: ME, metabolism
Swine

```
=> d que l10;d his l11

L5            1 SEA FILE=REGISTRY ABB=ON  15839-70-0
L6           84 SEA FILE=MEDLINE ABB=ON  L5
L9           47 SEA FILE=MEDLINE ABB=ON  L6 AND (CS OR BI OR IP)/CT
L10          20 SEA FILE=MEDLINE ABB=ON  L9 AND (MANNOSE# OR DEOXYMANNOSE?)



     (FILE 'MEDLINE' ENTERED AT 07:54:19 ON 13 NOV 2000)
L11           9 S L10 NOT L3

=> d .med 1-9 l11


L11  ANSWER 1 OF 9  MEDLINE
AN   2000035789     MEDLINE
DN   20035789
TI   Leukocyte adhesion deficiency type II.
AU   Becker D J; Lowe J B
CS   Cellular and Molecular Biology Program, Howard Hughes Medical Institute,
     Department of Pathology, University of Michigan Medical School, Ann Arbor
     48109-0650, USA.
NC   CA71931 (NCI)
     GM07863 (NIGMS)
SO   BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Oct 8) 1455 (2-3) 193-204.  Ref: 62
     Journal code: A0W. ISSN: 0006-3002.
CY   Netherlands
DT   Journal; Article; (JOURNAL ARTICLE)
     General Review; (REVIEW)
     (REVIEW, TUTORIAL)
LA   English
FS   Priority Journals; Cancer Journals
EM   200002
EW   20000204
AB   Leukocyte adhesion deficiency type II (LAD II) is a rare disorder
     characterized by recurrent infections, persistent leukocytosis, and
severe
     mental and growth retardation. LAD II neutrophils are deficient in
     expression of selectin ligand activity, and exhibit a correspondingly
     diminished ability to roll on endothelium and to traffic to inflammatory
     sites in vivo. LAD II patients exhibit a deficiency in the expression of
     cell surface fucosylated glycan structures that include the H and Lewis
     blood group determinants and the sialyl Lewis x epitope, yet the
     corresponding fucosyltransferase activities responsible for synthesis of
     these structures are expressed at normal levels. The molecular defect in
     LAD II has been localized to the pathway that synthesizes GDP-fucose from
     GDP-mannose. However, the two known component enzymes in this
     GDP-fucose biosynthetic pathway are normal in sequence and in expression
     levels in LAD II cells. The genetic lesion in LAD II that accounts for
the
     generalized fucosylation defect in LAD II patients remains to be
     determined.
CT   Check Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
      ABO Blood-Group System: CH, chemistry
```

ABO Blood-Group System: IM, immunology
Cell Adhesion Molecules: IM, immunology
Cell Line
Cell Movement
Fucose: ME, metabolism
Fucosyltransferases: ME, metabolism
Guanosine Diphosphate Fucose: BI, biosynthesis
Guanosine Diphosphate Mannose: ME, metabolism
Hydro-Lyases: AI, antagonists & inhibitors
Hydro-Lyases: ME, metabolism
*Leukocyte-Adhesion Deficiency Syndrome: GE, genetics
Leukocyte-Adhesion Deficiency Syndrome: IM, immunology
Leukocyte-Adhesion Deficiency Syndrome: ME, metabolism
Leukocytes: IM, immunology
Lewis Blood-Group System: CH, chemistry
Lewis Blood-Group System: IM, immunology
Lymphocytes: IM, immunology
Neutrophils: IM, immunology
Phenotype
Selectins: ME, metabolism

L11 ANSWER 2 OF 9 MEDLINE
AN 1999109950 MEDLINE
DN 99109950
TI The metabolism of 6-deoxyhexoses in bacterial and animal cells.
AU Tonetti M; Sturla L; Bisso A; Zanardi D; Benatti U; De Flora A
CS Institute of Biochemistry, University of Genova, Italy.
SO BIOCHIMIE, (1998 Nov) 80 (11) 923-31. Ref: 53
Journal code: A14. ISSN: 0300-9084.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199905
EW 19990504
AB L-fucose and L-rhamnose are two 6-deoxyhexoses naturally occurring in several complex carbohydrates. In prokaryotes both of them are found in polysaccharides of the cell wall, while in animals only L-fucose has been described, which mainly participates to the structure of glycoconjugates, either in the cell membrane or secreted in biological fluids, such as ABH blood groups and Lewis system antigens. L-fucose and L-rhamnose are synthesized by two de novo biosynthetic pathways starting from GDP-D-**mannose** and dTDP-D-glucose, respectively, which share several common features. The first step for both pathways is a dehydration reaction catalyzed by specific nucleotide-sugar dehydratases. This leads to the formation of unstable 4-keto-6-deoxy intermediates, which undergo a subsequent epimerization reaction responsible for the change from D- to L-conformation, and then a NADPH-dependent reduction of the 4-keto group, with the consequent formation of either GDP-L-fucose or dTDP-L-rhamnose. These compounds are then the substrates of specific glycosyltransferases which are responsible for insertion of either L-fucose or L-rhamnose in the corresponding glycoconjugates. The enzyme involved in the first step of GDP-L-fucose biosynthesis in E. coli, i.e., GDP-D-**mannose** 4,6 dehydratase, has been recently expressed as recombinant protein and

characterized in our laboratory. We have also cloned and fully characterized a human protein, formerly named FX, and an E. coli protein, WcaG, which display both the epimerase and the reductase activities, thus indicating that only two enzymes are required for GDP-L-fucose production.

Fucosylated complex glycoconjugates at the cell surface can then be recognized by specific counter-receptors in interacting cells, these mechanisms initiating important processes including inflammation and metastasis. The second pathway starting from dTDP-D-glucose leads to the synthesis of antibiotic glycosides or, alternatively, to the production of

dTDP-L-rhamnose. While several sets of data are available on the first enzyme of the pathway, i.e., dTDP-D-glucose dehydratase, the enzymes involved in the following steps still need to be identified and characterized.

CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Carbohydrate Epimerases: ME, metabolism
Fucose: BI, biosynthesis
*Fucose: ME, metabolism
Guanosine Diphosphate Fucose: ME, metabolism
Hydro-Lyases
Models, Chemical
Rhamnose: BI, biosynthesis
*Rhamnose: ME, metabolism

L11 ANSWER 3 OF 9 MEDLINE
AN 1998324808 MEDLINE
DN 98324808
TI Defective intracellular activity of GDP-D-**mannose** -4,6-dehydratase in leukocyte adhesion deficiency type II syndrome.
AU Sturla L; Etzioni A; Bisso A; Zanardi D; De Flora G; Silengo L; De Flora A; Tonetti M
CS Institute of Biochemistry, University of Genoa, Italy.
SO FEBS LETTERS, (1998 Jun 16) 429 (3) 274-8.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199810
EW 19981001
AB Leukocyte adhesion deficiency type II (LAD II) is a rare genetic disease characterized by severe immunodeficiency which is related to defective expression in leukocytes of sialyl-Lewis X (SLeX), a fucosylated ligand for endothelial selectins. The molecular basis of LAD II is still unknown,

but has been tentatively localized in the de novo pathway of GDP-L-fucose biosynthesis from GDP-D-**mannose**. Here, we demonstrate that in cell lysates from a LAD II patient, GDP-D-**mannose** -4,6-dehydratase (GMD), the first of the two enzymes of the pathway has a defective activity compared to control subjects. GMD in cell lysates from both parents showed intermediate activity levels. Cloning of GMD from patient and control lymphocytes ruled out any mutation affecting the amino

acid GMD sequence and the purified recombinant proteins from both controls

and the patient showed identical specific activities. Since the levels of

immunoreactive GMD in cell lysates were comparable in the patient and in controls, the biochemical deficiency of intracellular GMD activity in LAD II seems to be due to mutation(s) affecting some still unidentified GMD-regulating protein.

CT Check Tags: Case Report; Human; Male; Support, Non-U.S. Gov't
Cloning, Molecular
Guanosine Diphosphate Fucose: BI, biosynthesis
Guanosine Diphosphate Mannose: ME, metabolism
Hydro-Lyases: GE, genetics
*Hydro-Lyases: ME, metabolism
*Leukocyte-Adhesion Deficiency Syndrome: EN, enzymology
Leukocyte-Adhesion Deficiency Syndrome: GE, genetics
Oligosaccharides: BI, biosynthesis
Recombinant Proteins: ME, metabolism
RNA, Messenger: GE, genetics
Sequence Analysis, DNA

L11 ANSWER 4 OF 9 MEDLINE
AN 1998269129 MEDLINE
DN 98269129
TI Molecular cloning and expression of GDP-D-**mannose**-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells.
AU Ohyama C; Smith P L; Angata K; Fukuda M N; Lowe J B; Fukuda M
CS Glycobiology Program, La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037, USA.
NC R37 CA33000 (NCI)
P01 CA71932 (NCI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 5) 273 (23) 14582-7.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-AF040260
EM 199809
EW 19980903
AB Subsets of mammalian cell surface oligosaccharides contain specific fucosylated moieties expressed in lineage- and/or temporal-specific patterns. The functional significance of these fucosylated structures is incompletely defined, although there is evidence that subsets of them, represented by the sialyl Lex determinant, are important participants in leukocyte adhesion and trafficking processes. Genetic deletion of these fucosylated structures in the mouse has been a powerful tool to address functional questions about fucosylated glycans. However, successful use of such approaches can be problematic, given the substantial redundancy in the mammalian alpha-1,3-fucosyltransferase and alpha-1,2-fucosyltransferase gene families. To circumvent this problem, we have chosen to clone the genetic locus encoding a mammalian GDP-D-**mannose**-4,6-dehydratase (GMD). This enzyme generates GDP-**mannose**-4-keto-6-D-**deoxymannose** from GDP-**mannose**, which is then converted by the FX protein (GDP-4-keto-6-D-**deoxymannose** epimerase/GDP-4-keto-6-L-galactose reductase) to GDP-L-fucose. GMD is thus imperative for the synthesis of all fucosylated oligosaccharides. An expression cloning approach and the GMD-deficient CHO

host cell line Lec13 were used to generate a population of cDNA molecules enriched in GMD cDNAs. This enriched plasmid population was then screened using a human expressed sequence tag (EST AA065072) with sequence similarity to an Arabidopsis thaliana GMD cDNA. This approach, together with 5'-rapid amplification of cDNA ends, yielded a human cDNA that complements the fucosylation defect in the Lec13 cell line. Northern blot analyses indicate that the GMD transcript is absent in Lec13 cells, confirming the genetic deficiency of this locus in these cells. By contrast, the transcript encoding the FX protein, which forms GDP-L-fucose from the ketosugar intermediate produced by GMD, is present in increased amounts in the Lec13 cells. These results suggest that metabolites generated in this pathway may participate in the transcriptional regulation of the FX protein and possibly the GMD protein. The results also suggest that the genomic structure encoding GMD in Lec13 cells likely has a defect different from a point mutation in the coding region.

CT Check Tags: Animal; Human; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
Base Sequence
Carbohydrate Epimerases: GE, genetics
Cell Line
Cloning, Molecular
CHO Cells
Fluorescent Antibody Technique
*Fucose: ME, metabolism
Guanosine Diphosphate Fucose: BI, biosynthesis
Guanosine Diphosphate Mannose: ME, metabolism
Hamsters
*Hydro-Lyases: CH, chemistry
Hydro-Lyases: DF, deficiency
Molecular Sequence Data
Molecular Structure
RNA, Messenger: ME, metabolism
Sequence Analysis, DNA
Sugar Alcohol Dehydrogenases: GE, genetics

L11 ANSWER 5 OF 9 MEDLINE
AN 1998119777 MEDLINE
DN 98119777
TI Abnormal synthesis of **mannose** 1-phosphate derived carbohydrates in carbohydrate-deficient glycoprotein syndrome type I fibroblasts with phosphomannomutase deficiency.
AU Korner C; Lehle L; von Figura K
CS Georg-August-Universitat, Abt. Biochemie II, Gottingen, Germany.
SO GLYCOBIOLOGY, (1998 Feb) 8 (2) 165-71.
Journal code: BEL. ISSN: 0959-6658.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199805
EW 19980502
AB In fibroblasts from five patients with carbohydrate-deficient glycoprotein syndrome type 1, the incorporation of [2-3H] **mannose** into **mannose** phosphates, GDP-**mannose**, GDP-fucose, dolichol-P-

mannose, lipid-linked oligosaccharides, and glycoprotein fraction was determined. We observed a 3- to 5-fold reduction of incorporation of radioactivity into **mannose** 1-phosphate, GDP-**mannose**, GDP-fucose, dolichol-P-**mannose**, and nascent glycoproteins. The incorporation of radioactivity into **mannose** 6-phosphate was normal. The formation of lipid linked oligosaccharides was only slightly affected (</=20%), but their size was severely reduced, mostly containing five or fewer residues. As a consequence, truncated oligosaccharides were transferred to newly synthesized glycoproteins. The metabolic changes can be explained by a deficiency of phosphomannomutase activity, which was reduced to </=10% of control.

CT Check Tags: Human; Support, Non-U.S. Gov't
*Carbohydrate-Deficient Glycoprotein Syndrome: EN, enzymology
*Carbohydrate-Deficient Glycoprotein Syndrome: ME, metabolism
Carbohydrate-Deficient Glycoprotein Syndrome: PA, pathology
***Carbohydrates: BI, biosynthesis**
Carbohydrates: DF, deficiency
Cells, Cultured
Dolichol Monophosphate Mannose: ME, metabolism
Fibroblasts: EN, enzymology
Fibroblasts: ME, metabolism
Guanosine Diphosphate Fucose: BI, biosynthesis
Guanosine Diphosphate Mannose: BI, biosynthesis
Lipopolysaccharides: BI, biosynthesis
Mannosephosphates: BI, biosynthesis
*Mannosephosphates: ME, metabolism
*Phosphotransferases (Phosphomutases): DF, deficiency

L11 ANSWER 6 OF 9 MEDLINE
AN 97066899 MEDLINE
DN 97066899
TI Synthesis of GDP-L-fucose by the human FX protein.
AU Tonetti M; Sturla L; Bisso A; Benatti U; De Flora A
CS Institute of Biochemistry, University of Genova, and Advanced Biotechnology Center, Viale Benedetto XV, 1, 16132 Genova, Italy.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Nov 1) 271 (44) 27274-9. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-U58766
EM 199702
EW 19970204
AB FX is a homodimeric NADP(H)-binding protein of 68 kDa, first identified in human erythrocytes, from which it was purified to homogeneity. Its function has been unrecognized despite partial structural and genetic characterization. Recently, on the basis of partial amino acid sequence, it proved to be the human homolog of the murine protein P35B, a tumor rejection antigen. In order to address the biochemical role of FX, its primary structure was completed by cDNA sequencing. This sequence revealed a significant homology with many proteins from different organisms. Specifically, FX showed a remarkable similarity with a putative Escherichia coli protein, named Yefb, whose gene maps in a region of E. coli chromosome coding for enzymes involved in synthesis and utilization

of GDP-D-**mannose**. Accordingly, a possible role of FX in this metabolism was investigated. The data obtained indicate FX as the enzyme responsible for the last step of the major metabolic pathway resulting in GDP-L-fucose synthesis from GDP-D-**mannose** in procaryotic and eucaryotic cells. Specifically, purified FX apparently catalyzes a combined epimerase and NADPH-dependent reductase reaction, converting GDP-4-keto-6-D-**deoxymannose** to GDP-L-fucose. This is the substrate of several fucosyltranferases involved in the correct expression of many glyconjugates, including blood groups and developmental antigens.

CT Check Tags: Animal; Comparative Study; Human; Support, Non-U.S. Gov't
Amino Acid Sequence
Antigens, Surface: CH, chemistry
Base Sequence
Blood Proteins: CH, chemistry
Blood Proteins: ME, metabolism
Caenorhabditis elegans
Carcinoma, Hepatocellular
Carrier Proteins: BI, biosynthesis
Carrier Proteins: CH, chemistry
*Carrier Proteins: ME, metabolism
Cell Line
Chromatography, High Pressure Liquid
Cytosol: ME, metabolism
DNA, Complementary
*Erythrocytes: ME, metabolism
Escherichia coli
*Guanosine Diphosphate Fucose: ME, metabolism
Guanosine Diphosphate Mannose: ME, metabolism
Liver Neoplasms
Mice
Molecular Sequence Data
Molecular Structure
Nerve Tissue Proteins: CH, chemistry
Polymerase Chain Reaction
Sequence Homology, Amino Acid

L11 ANSWER 7 OF 9 MEDLINE
AN 96060831 MEDLINE
DN 96060831
TI Sequence and analysis of the O antigen gene (rfb) cluster of Escherichia coli O111.
AU Bastin D A; Reeves P R
CS Department of Microbiology, University of Sydney, New South Wales, Australia.
SO GENE, (1995 Oct 16) 164 (1) 17-23.
Journal code: FOP. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U13629
EM 199602
AB The O antigens found in Salmonella enterica (Se) and Escherichia coli (Ec) show a great deal of diversity, and only three structures are known to be common to both genera. Two of them contain the 3,6-dideoxyheoxse colitose,

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FROM JANUARY 1969 TO DATE.

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     (FILE 'MEDLINE' ENTERED AT 07:54:19 ON 13 NOV 2000)
               DEL HIS Y

     FILE 'REGISTRY' ENTERED AT 08:00:24 ON 13 NOV 2000
L1             1 S 15839-70-0
L2             1 S 18186-48-6

     FILE 'BIOSIS' ENTERED AT 08:00:51 ON 13 NOV 2000
L3           228 S L1  OR  GDP FUCOSE OR GUANOSINE (2W) DIPHOS? FUCOSE
L4             0 S GKDM
L5             6 S GDP (5A) KETO (5A) (DEOXYMANNOSE? OR DEOXY MANNOSE OR DE
OXY
L6           725 S (GUANOSINE OR GDP ) (5A) MANNOSE
L7             4 S L3 AND L5
L8           282 S L1  OR  GDP(2W) FUCOSE OR GUANOSINE (2W) DIPHOS? (2W) FUCOSE
L9             5 S L8 AND L5
L10           14 S GDP (5A) KETO (5A) (DEOXYMANNOSE? OR DEOXY(2W) MANNOSE OR
DE
L11           11 S L10 AND L8
L12           11 S L11 OR L9 OR L7

     FILE 'BIOSIS' ENTERED AT 08:09:08 ON 13 NOV 2000

=> d bib ab it 1-11


L12  ANSWER 1 OF 11  BIOSIS  COPYRIGHT 2000 BIOSIS
AN   2000:465954  BIOSIS
DN   PREV200000465954
TI   Functional expression of Escherichia coli enzymes synthesizing GDP
     -L-fucose from inherent GDP-D-mannose in Saccharomyces
     cerevisiae.
AU   Mattila, Pirkko (1); Rabina, Jarkko; Hortling, Solveig; Helin, Jari;
     Renkonen, Risto
CS   (1) Department of Bacteriology and Immunology, University of Helsinki,
     Haartmaninkatu 3, FIN-00014, Helsinki Finland
SO   Glycobiology, (October, 2000) Vol. 10, No. 10, pp. 1041-1047. print.
     ISSN: 0959-6658.
DT   Article
```

LA English
SL English
AB Fucosylation of glycans on glycoproteins and -lipids requires the enzymatic activity of relevant fucosyltransferases and **GDP**-L-**fucose** as the donor. Due to the biological importance of fucosylated glycans, a readily accessible source of **GDP**-L-**fucose** would be required. Here we describe the construction of a stable recombinant S.cerevisiae strain expressing the E.coli genes gmd and wcaG encoding the two enzymes, GDP-mannose-4,6-dehydratase (GMD) and **GDP**-4-**keto**-6-**deoxy**-D-**mannose** -3,5-epimerase/4-reductase (GMER(FX)) respectively, needed to convert GDP-mannose to **GDP-fucose** via the de novo pathway. Taking advantage of the rich inherent cytosolic GDP-mannose pool in S.cerevisiae cells we could easily produce 0.2 mg/l of **GDP**-L-**fucose** with this recombinant yeast strain without addition of any external GDP-mannose. The **GDP**-L-**fucose** product could be used as the fucose donor for alpha1,3fucosyltransferase to synthesize sialyl Lewis x (sLex), a glycan crucial for the selectin-dependent leukocyte traffic.
IT Major Concepts
Biochemistry and Molecular Biophysics; Immune System (Chemical Coordination and Homeostasis)
IT Parts, Structures, & Systems of Organisms
leukocyte: blood and lymphatics, immune system, selectin-dependent traffic
IT Chemicals & Biochemicals
Escherichia coli enzymes: functional expression; GDP-D-mannose: inherent; **GDP**-L-**fucose**: synthesis
ORGN Super Taxa
Ascomycetes: Fungi, Plantae; Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Escherichia coli (Enterobacteriaceae); Saccharomyces cerevisiae (Ascomycetes)
ORGN Organism Superterms
Bacteria; Eubacteria; Fungi; Microorganisms; Nonvascular Plants; Plants
RN 3123-67-9 (GDP-D-MANNOSE)
15839-70-0 (**GDP**-L-**FUCOSE**)

L12 ANSWER 2 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 2000:419024 BIOSIS
DN PREV200000419024
TI Preparative synthesis of **GDP**-beta-L-**fucose** by recombinant enzymes from enterobacterial sources.
AU Albermann, Christoph; Distler, Juergen; Piepersberg, Wolfgang (1)
CS (1) Chemische Mikrobiologie, Bergische Universitaet GH Wuppertal, Gauss-Strasse 20, FB9, D-42097, Wuppertal Germany
SO Glycobiology, (September, 2000) Vol. 10, No. 9, pp. 875-881. print. ISSN: 0959-6658.
DT Article
LA English
SL English
AB The 6-deoxyhexose L-fucose is an important and characteristic element in glycoconjugates of bacteria (e.g., lipopolysaccharides), plants (e.g., xyloglucans) and animals (e.g., glycolipids, glycoproteins, and

oligosaccharides). The biosynthetic pathway of **GDP**-L-**fucose** starts with a dehydration of GDP-D-mannose catalyzed by GDP-D-mannose 4,6-dehydratase (Gmd) creating **GDP**-4-**keto**-6-**deoxymannose** which is subsequently converted by the **GDP**-4-**keto**-6-**deoxy**-D-**mannose** 3-5,-epimerase-4-reductase (WcaG; **GDP**-beta-L-**fucose** synthetase) to **GDP**-beta-L-**fucose**. Both biosynthetic genes gmd and wcaG were cloned from Escherichia coli K12 and the enzymes overexpressed under control of the T7 promoter in the expression vectors pET11a and pET16b, yielding both native and N-terminal His-tag fusion proteins, respectively. The activities of the Gmd and WcaG were analyzed. The enzymatic conversion from GDP-D-mannose to **GDP**-beta-L-**fucose** was optimized and the final product was purified. The formation of **GDP**-beta-L-**fucose** by the recombinant enzymes was verified by HPLC and NMR analyses. The His-tag fusion variants of the Gmd and WcaG proteins were purified to near homogeniety. The His-tag Gmd recombinant enzyme was inactive, whereas His-tag WcaG showed very similar enzymatic properties relative to the native **GDP**-beta-L-**fucose** synthetase. With the purified His-tag WcaG Km and Vmax values, respectively, of 40 muM and 23 nkat/mg protein for the substrate **GDP**-4-**keto**-6-**deoxy**-D-**mannose** and of 21 muM and 10 nkat/mg protein for the cosubstrate NADPH were obtained; a pH optimum of 7.5 was determined and the enzyme was stimulated to equal extend by the divalent cations Mg2+ and Ca2+. The Gmd enzyme showed a strong feedback inhibition by **GDP**-beta-L-**fucose**.

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
6-deoxyhexose-L-fucose; GDP-D-mannose 4,6-dehydratase: activity, cloning, overproduction; **GDP**-L-**fucose** synthetase: activity, cloning, overproduction; **GDP**-beta-L-**fucose**: purification, synthesis

IT Methods & Equipment
HPLC [high performance liquid chromatography]: analytical method, liquid chromatography; NMR spectroscopy: analytical method, spectroscopic techniques: CB

ORGN Super Taxa
Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms

ORGN Organism Name
Escherichia coli (Enterobacteriaceae): strain-K-12

ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms

RN 37211-59-9 (GDP-D-MANNOSE 4,6-DEHYDRATASE)
15839-70-0 (**GDP**-BETA-L-**FUCOSE**)

L12 ANSWER 3 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 2000:129463 BIOSIS
DN PREV200000129463
TI Structural and kinetic analysis of Escherichia coli GDP-mannose 4,6 dehydratase provides insights into the enzyme's catalytic mechanism and regulation by **GDP-fucose**.
AU Somoza, John R.; Menon, Saurabh; Schmidt, Holly; Joseph-McCarthy, Diane;

Dessen, Andrea; Stahl, Mark L.; Somers, William S.; Sullivan, Francis X. (1)
CS (1) Wyeth Research, 87 Cambridgepark Drive, Cambridge, MA, 02140 USA
SO Structure (London)., (Feb., 2000) Vol. 8, No. 2, pp. 123-135. ISSN: 0969-2126.
DT Article
LA English
SL English
AB Background: GDP-mannose 4,6 dehydratase (GMD) catalyzes the conversion of **GDP**-(D)-mannose to **GDP**-4-**keto**, 6-**deoxy**-(D)-**mannose**. This is the first and regulatory step in the de novo biosynthesis of **GDP**-(L)-**fucose**. **Fucose** forms part of a number of glycoconjugates, including the ABO blood groups and the selectin ligand sialyl Lewis X. Defects in **GDP-fucose** metabolism have been linked to leukocyte adhesion deficiency type II (LADII). Results: The structure of the GDP-mannose 4,6 dehydratase apo enzyme has been determined and refined using data to 2.3 ANG resolution. GMD is a homodimeric protein with each monomer composed of two domains. The larger N-terminal domain binds the NADP(H) cofactor in a classical Rossmann fold and the C-terminal domain harbors the sugar-nucleotide binding site. We have determined the GMD dissociation constants for NADP, NADPH and GDP-mannose. Each GMD monomer binds one cofactor and one substrate molecule, suggesting that both subunits are catalytically competent. **GDP-fucose** acts as a competitive inhibitor, suggesting that it binds to the same site as GDP-mannose, providing a mechanism for the feedback inhibition of fucose biosynthesis. Conclusions: The X-ray structure of GMD reveals that it is a member of the short-chain dehydrogenase/reductase (SDR) family of proteins. We have modeled the binding of NADP and GDP-mannose to the enzyme and mutated four of the active-site residues to determine their function. The combined modeling and mutagenesis data suggests that at position 133 threonine substitutes serine as part of the serine-tyrosine-lysine catalytic triad common to the SDR family and Glu135 functions as an active-site base.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Metabolism
IT Chemicals & Biochemicals
GDP fucose; GDP-mannose 4,6 dehydratase; GMD; NADP; NADPH; fucose: biosynthesis
IT Methods & Equipment
kinetic analysis: activity assays, analytical method
ORGN Super Taxa
Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Escherichia coli (Enterobacteriaceae)
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms
RN **15839-70-0 (GDP FUCOSE)**
37211-59-9 (GDP-MANNOSE 4,6 DEHYDRATASE)
53-59-8 (NADP)
53-57-6 (NADPH)
2438-80-4 (FUCOSE)

L12 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:86414 BIOSIS
DN PREV199900086414
TI **GDP-fucose** synthetase from Escherichia coli: Structure of a unique member of the short-chain dehydrogenase/reductase family that catalyzes two distinct reactions at the same active site.
AU Somers, William S.; Stahl, Mark L.; Sullivan, Francis X. (1)
CS (1) Small Mol. Drug Discovery, Genet. Inst. Inc., 87 Cambridgepark Drive, Cambridge, MA 02140 USA
SO Structure (London), (Dec. 15, 1998) Vol. 6, No. 12, pp. 1601-1612. ISSN: 0969-2126.
DT Article
LA English
AB Background: In all species examined, **GDP-fucose** is synthesized from GDP-mannose in a three-step reaction catalyzed by two enzymes, GDP-mannose 4,6 dehydratase and a dual function 3,5-epimerase-4-reductase named **GDP-fucose** synthetase. In this latter aspect fucose biosynthesis differs from that of other deoxy and dideoxy sugars, in which the epimerase and reductase activities are present as separate enzymes. Defects in **GDP-fucose** biosynthesis have been shown to affect nodulation in bacteria, stem development in plants, and are associated with the immune defect leukocyte adhesion deficiency type II in humans. Results: We have determined the structure of **GDP-fucose** synthetase from Escherichia coli at 2.2 ANG resolution. The structure of **GDP-fucose** synthetase is closely related to that of UDP-galactose 4-epimerase and more distantly to other members of the short-chain dehydrogenase/reductase family. We have also determined the structures of the binary complexes of **GDP-fucose** synthetase with its substrate NADPH and its product NADP+. The nicotinamide cofactors bind in the syn and anti conformations, respectively. Conclusions: **GDP-fucose** synthetase binds its substrate, NADPH, in the proper orientation (syn) for transferring the 4-pro-S hydride of the nicotinamide. We have observed a single binding site in **GDP-fucose** synthetase for the second substrate, **GDP-4-keto,6-deoxy-mannose**. This implies that both the epimerization and reduction reactions occur at the same site in the enzyme. As is the case for all members of the short-chain family of dehydrogenase/reductases, **GDP-fucose** synthetase retains the Ser-Tyr-Lys catalytic triad. We propose that this catalytic triad functions in a mechanistically equivalent manner in both the epimerization and reduction reactions. Additionally, the X-ray structure has allowed us to identify other residues that are potentially required for substrate binding and catalysis.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
Escherichia coli **GDP-fucose** synthetase: active site, short-chain dehydrogenase/reductase, structure
IT Sequence Data
1BSV: Brookhaven Protein Data Bank, amino acid sequence; 1FXS: Brookhaven Protein Data Bank, amino acid sequence; 1GFS: Brookhaven Protein Data Bank, amino acid sequence

RN 9035-82-9 (DEHYDROGENASE)
9037-80-3 (REDUCTASE)

L12 ANSWER 5 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:35636 BIOSIS
DN PREV199900035636
TI **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase from Escherichia coli, a key enzyme in the biosynthesis of **GDP**-L-**fucose**, displays the structural characteristics of the RED protein homology superfamily.
AU Rizzi, Menico; Tonetti, Michela; Vigevani, Pierpaolo; Sturla, Laura; Bisso, Angela; De Flora, Antonio; Bordo, Domenico; Bolognesi, Martino (1)
CS (1) Centro Biotecnol. Avanzate-IST, Dip. Fis.-INFM, Univ. Genova, Largo Rosanna Benzi 10, 16132 Genova Italy
SO Structure (London), (Nov. 15, 1998) Vol. 6, No. 11, pp. 1453-1465. ISSN: 0969-2126.
DT Article
LA English
AB Background: The process of **guanosine** 5'-**diphosphate** L-**fucose** (**GDP**-L-**fucose**) biosynthesis is conserved throughout evolution from prokaryotes to man. In animals, **GDP**-L-**fucose** is the substrate of fucosyltransferases that participate in the biosynthesis and remodeling of glycoconjugates, including ABH blood group and Lewis-system antigens. The 'de novo' pathway of **GDP**-L-**fucose** biosynthesis from GDP-D-mannose involves a GDP-o-mannose 4,6 dehydratase (GMD) and a **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase (GMER). Neither of the catalytic mechanisms nor the three-dimensional structures of the two enzymes has been elucidated yet. The severe leukocyte adhesion deficiency (LAD)' type II genetic syndrome is known to result from deficiencies in this de novo pathway. Results: The crystal structures of apo- and holo-GMER have been determined at 2.1 ANG and 2.2 ANG resolution, respectively. Each subunit of the homodimeric (2 X 34 kDa) enzyme is composed of two domains. The N-terminal domain, a six-stranded Rossmann fold, binds NADP+; the C-terminal domain (about 100 residues) displays an alpha/beta topology. NADP+ interacts with residues Arg12 and Arg36 at the adenylic ribose phosphate; moreover, a protein loop based on the Gly-X-X-Gly-X-X-Gly motif (where X is any amino acid) stabilizes binding of the coenzyme diphosphate bridge. The nicotinamide and the connected ribose ring are located close to residues Ser107, Tyr136 and Lys140, the putative GMER active-site center. Conclusions: The GMER fold is reminiscent of that observed for UDP-galactose epimerase (UGE) from Escherichia coli. Consideration of the enzyme fold and of its main structural features allows assignment of GMER to the reductase-epimerase-dehydrogenase (RED) enzyme homology superfamily, to which short-chain dehydrogenase/reductases (SDRs) also belong. The location of the NADP+ nicotinamide ring at an interdomain cleft is compatible with substrate binding in the C-terminal domain.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
Escherichia coli **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase: reductase-epimerase dehydrogenase protein homology superfamily member, structure; **GDP**-L-**fucose**: biosynthesis

RN 37342-00-0 (EPIMERASE)
9037-80-3 (REDUCTASE)
15839-70-0 (**GDP**-L-**FUCOSE**)
9035-82-9 (DEHYDROGENASE)

L12 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:390115 BIOSIS
DN PREV199800390115
TI Preliminary crystallographic investigations of recombinant **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase from E. coli.
AU Tonetti, M. (1); Rizzi, M.; Vigevani, P.; Sturla, L. (1); Bisso, A. (1); De Flora, A.; Bolognesi, M.
CS (1) Ist. Policattedra Chimica Biologica, Univ. Genova, Viale Benedetto XV, 1, I16132 Genova Italy
SO Acta Crystallographica Section D Biological Crystallography, (July 1, 1998) Vol. 54, No. 4, pp. 684-686.
ISSN: 0907-4449.
DT Article
LA English
AB The **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase (GM-ER) isolated from E. coli has been overexpressed as a GST-fusion protein and purified to homogeneity. The enzyme, an NADP+(H)-binding homodimer of 70 kDa, is responsible for the production of **GDP**-L-**fucose**. GM-ER shows significant structural homology to the human erythrocyte protein FX, which is involved in blood-group glycoconjugate biosynthesis, displaying 3,5 epimerase/reductase activity on **GDP**-4-**keto**-6-**deoxy**-D-**mannose**. GM-ER has been crystallized in a trigonal crystalline form, containing one molecule per asymmetric unit, suitable for high-resolution crystallographic investigations.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques
IT Chemicals & Biochemicals
recombinant **GDP**-4-**keto**-6-**deoxy**-D-**mannose** epimerase/reductase: analysis, structure
IT Methods & Equipment
crystallography: analytical method, crystallographic techniques
ORGN Super Taxa
Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
E. coli [Escherichia-coli] (Enterobacteriaceae)
ORGN Organism Superterms
Bacteria; Eubacteria; Microorganisms
RN 9037-80-3 (REDUCTASE)

L12 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:301070 BIOSIS
DN PREV199800301070
TI Molecular cloning and expression of GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells.
AU Ohyama, Chikara; Smith, Peter L.; Angata, Kiyohiko; Fukuda, Michiko N.; Lowe, John B.; Fukuda, Minoru (1)

CS (1) Burnham Inst., 10901 N. Torrey Pines Rd., La Jolla, CA 92037 USA
SO Journal of Biological Chemistry, (June 5, 1998) Vol. 273, No. 23, pp. 14582-14587.
ISSN: 0021-9258.
DT Article
LA English
AB Subsets of mammalian cell surface oligosaccharides contain specific fucosylated moieties expressed in lineage- and/or temporal-specific patterns. The functional significance of these fucosylated structures is incompletely defined, although there is evidence that subsets of them, represented by the sialyl Lex determinant, are important participants in leukocyte adhesion and trafficking processes. Genetic deletion of these fucosylated structures in the mouse has been a powerful tool to address functional questions about fucosylated glycans. However, successful use of such approaches can be problematic, given the substantial redundancy in the mammalian alpha-1,3-fucosyltransferase and alpha-1,2-fucosyltransferase gene families. To circumvent this problem, we have chosen to clone the genetic locus encoding a mammalian GDP-D-mannose-4,6-dehydratase (GMD). This enzyme generates **GDP**-mannose-4-**keto**-6-D-**deoxymannose** from **GDP**-mannose, which is then converted by the FX protein. (**GDP**-4-**keto**-6-D-**deoxymannose** epimerase/**GDP**-4-**keto**-6-L-galactose reductase) to **GDP**-L-**fucose**. GMD is thus imperative for the synthesis of all fucosylated oligosaccharides. An expression cloning approach and the GMD-deficient CHO host cell line Lec13 were used to generate a population of cDNA molecules enriched in GMD cDNAs. This enriched plasmid population was then screened using a human expressed sequence tag (EST AA065072) with sequence similarity to an Arabidopsis thaliana GMD cDNA. This approach, together with 5'-rapid amplification of cDNA ends, yielded a human cDNA that complements the fucosylation defect in the Lec13 cell line. Northern blot analyses indicate that the GMD transcript is absent in Lec13 cells, confirming the genetic deficiency of this locus in these cells. By contrast, the transcript encoding the FX protein, which forms **GDP**-L-**fucose** from the ketosugar intermediate produced by GMD, is present in increased amounts in the Lec13 cells. These results suggest that metabolites generated in this pathway may participate in the transcriptional regulation of the FX protein and possibly the GMD protein.
The results also suggest that the genomic structure encoding GMD in Lec13 cells likely has a defect different from a point mutation in the coding region.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Metabolism
IT Chemicals & Biochemicals
complementary DNA; fucose: metabolism; GDP-D-mannose-4,6-dehydratase: cloning, expression
IT Sequence Data
AF040260: EBI, GenBank, amino acid sequence, nucleotide sequence
IT Methods & Equipment
in vitro enzyme assay: methodological approach; Northern blotting: analysis/characterization techniques, analytical method
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name

Lec13 (Cricetidae)
ORGN Organism Superterms
Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates
RN 2438-80-4 (FUCOSE)

L12 ANSWER 8 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:226493 BIOSIS
DN PREV199800226493
TI Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of **GDP-fucose** biosynthesis in vitro.
AU Sullivan, Francis X. (1); Kumar, Ravindra; Kriz, Ronald; Stahl, Mark; Xu, Guang-Yi; Rouse, Jason; Chang, Xiao-Jia; Boodhoo, Amechand; Potvin, Barry;
Cumming, Dale A.
CS (1) Small Mol. Drug Discovery, Genet. Inst. Inc., 87 Cambridgepark Dr., Cambridge, MA 02140 USA
SO Journal of Biological Chemistry, (April 3, 1998) Vol. 273, No. 14, pp. 8193-8202.
ISSN: 0021-9258.
DT Article
LA English
AB We have cloned the cDNA encoding human GDP-mannose 4,6-dehydratase, the first enzyme in the pathway converting GDP-mannose to **GDP-fucose**. The message is expressed in all tissues and cell lines examined, and the cDNA complements Lec13, a Chinese Hamster Ovary cell line deficient in GDP-mannose 4,6-dehydratase activity. The human GDP-mannose 4,6-dehydratase polypeptide shares 61% identity with the enzyme from Escherichia coli, suggesting broad evolutionary conservation. Purified recombinant enzyme utilizes NADP+ as a cofactor and, like its E. coli counterpart, is inhibited by **GDP-fucose**, suggesting that this aspect of regulation is also conserved. We have isolated the product of the dehydratase reaction, **GDP-4-keto-6-deoxymannose**, and confirmed its structure by electrospray ionization-mass spectrometry and high field NMR. Using purified recombinant human GDP-mannose 4,6-dehydratase and FX protein (**GDP-keto-6-deoxymannose** 3,5-epimerase, 4-reductase), we show that the two proteins alone are sufficient to convert GDP-mannose to **GDP-fucose** in vitro. This unequivocally demonstrates that the epimerase and reductase activities are on a single polypeptide. Finally, we show that the two homologous enzymes from E. coli are sufficient to carry out the same enzymatic pathway in bacteria.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
human GDP-mannose 4,6-dehydratase: analysis, expression, isolation, sequence homology, purification, molecular cloning; **GDP-fucose**: in vitro biosynthesis; **GDP-keto-6-deoxymannose** 3,5-epimerase, 4-reductase: expression, fusion protein, purification; GDP-mannose; NADP: enzyme cofactor
IT Sequence Data
AF042377: EBI, GenBank, nucleotide sequence, amino acid sequence
IT Methods & Equipment
electrospray ionization-mass spectrometry: analysis/characterization techniques, spectroscopic techniques, analytical method; gene cloning:

methodological approach; high field NMR: analysis/characterization techniques, analytical method, spectroscopic techniques; in vitro assay: methodological approach; paper chromatography: chromatographic techniques, isolation method
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Enterobacteriaceae: Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms
ORGN Organism Name
Escherichia-coli (Enterobacteriaceae); Lec13 (Cricetidae)
ORGN Organism Superterms
Animals; Bacteria; Chordates; Eubacteria; Mammals; Microorganisms; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates
RN **15839-70-0** (**GDP-FUCOSE**)
53-59-8 (NADP)
3123-67-9 (GDP-MANNOSE)

L12 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:571096 BIOSIS
DN PREV199799285777
TI Synthesis of **GDP**-L-**fucose** by the human FX protein.
AU Tonetti, Michela (1); Sturla, Laura; Bisso, Angela; Benatti, Umberto; De Flora, Antonio
CS (1) Inst. Biochemistry, Univ. Genova, Viale Benedetto XV 1, 16132 Genova Italy
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 44, pp. 27274-27279.
ISSN: 0021-9258.
DT Article
LA English
AB FX is a homodimeric NADP(H)-binding protein of 68 kDa, first identified in
human erythrocytes, from which it was purified to homogeneity. Its function has been unrecognized despite partial structural and genetic characterization. Recently, on the basis of partial amino acid sequence, it proved to be the human homolog of the murine protein P35B, a tumor rejection antigen. In order to address the biochemical role of FX, its primary structure was completed by cDNA sequencing. This sequence revealed
a significant homology with many proteins from different organisms. Specifically, FX showed a remarkable similarity with a putative Escherichia coli protein, named Yefb, whose gene maps in a region of E. coli chromosome coding for enzymes involved in synthesis and utilization of GDP-D-mannose. Accordingly, a possible role of FX in this metabolism was investigated. The data obtained indicate FX as the enzyme responsible for the last step of the major metabolic pathway resulting in **GDP**-L-**fucose** synthesis from GDP-D-mannose in procaryotic and eucaryotic cells. Specifically, purified FX apparently catalyzes a combined epimerase and NADPH-dependent reductase reaction, converting **GDP**-4-**keto**-6-D-**deoxymannose** to **GDP**-L-**fucose**. This is the substrate of several fucosyltranferases involved in the correct expression of many glyconjugates, including blood groups and developmental antigens.
IT Major Concepts
Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation)
IT Chemicals & Biochemicals

GDP-L-**FUCOSE**; GENBANK-U58766
IT Sequence Data
amino acid sequence; molecular sequence data; nucleotide sequence; EBI-U58766; GENBANK-U58766
IT Miscellaneous Descriptors
BIOCHEMISTRY AND BIOPHYSICS; BLOOD AND LYMPHATICS; BLOOD GROUPS; DEVELOPMENTAL ANTIGEN; ERYTHROCYTE; **GDP**-L-**FUCOSE**; HUMAN FX PROTEIN
RN **15839-70-0** (**GDP**-L-**FUCOSE**)
177823-39-1 (GENBANK-U58766)

L12 ANSWER 10 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1993:185352 BIOSIS
DN PREV199395095802
TI Evidence that the enzyme catalyzing the conversion of guanosine diphosphate D-mannose to a 4-keto sugar nucleotide intermediate requires nicotinamide adenine dinucleotide phosphate.
AU Yamamoto, Kenji (1); Katayama, Isao; Onoda, Yukiko; Inami, Masaki; Kumagai, Hidehiko; Tochikura, Tatsurokuro
CS (1) Dep. Food Sci. and Technol., Fac. Agriculture, Kyoto University, Sakyo-ku, Kyoto 606 Japan
SO Archives of Biochemistry and Biophysics, (1993) Vol. 300, No. 2, pp. 694-698.
ISSN: 0003-9861.
DT Article
LA English
AB The first enzyme in the formation of **GDP**-L-**fucose** from GDP-D-mannose, which forms a GDP-4-keto sugar intermediate, was purified to homogeneity from cell extracts of Klebsiella pneumoniae. During purification, the enzyme was found to be highly activated by NADP. It was proven that the pyridine nucleotide coenzyme of the enzyme was NADP, not NAD, which differs from previously accepted information. NAD had not effect on enzyme activity. The product of the enzyme reaction with NADP as
coenzyme was separated from other nucleotides by high-performance liquid chromatography, and using ion spray liquid chromatography/mass spectrometry the mass was determined for the first time, as 587, which is same as the calculated mass of **GDP**-4-**keto**-6-**deoxy**-D-**mannose**.
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Metabolism; Physiology
IT Chemicals & Biochemicals
NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE; NADP
IT Miscellaneous Descriptors
NADP; PURIFICATION METHOD
ORGN Super Taxa
Enterobacteriaceae: Eubacteria, Bacteria
ORGN Organism Name
Klebsiella pneumoniae (Enterobacteriaceae)
ORGN Organism Superterms
bacteria; eubacteria; microorganisms
RN 53-59-8 (NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE)
53-59-8 (NADP)

L12 ANSWER 11 OF 11 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1988:154410 BIOSIS

DN BA85:78063
TI AN EPIMERASE-REDUCTASE IN L FUCOSE SYNTHESIS.
AU CHANG S; DUERR B; SERIF G
CS DEP. BIOCHEM., OHIO STATE UNIV., 484 W. 12TH AVE., COLUMBUS, OHIO 43210-1292.
SO J BIOL CHEM, (1988) 263 (4), 1693-1697.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB The first committed enzyme in **GDP**-L-**fucose** formation from GDP-D-mannose is GDP-D-mannose 4,6-dehydratase, which forms **GDP**-4-**keto**-6-**deoxy**-D-**mannose**. The uncertain enzymatic steps beyond this point were examined in this study. Assays were developed for the epimerase and reductase activities which the putative pathway would predict. A protein was isolated exhibiting homogeneity by several criteria. This single protein, which forms **GDP**-L-fucrose from **GDP**-4-**keto**-6-**deoxy**-D-**mannose** and NADH, appears to possess both epimerase and reductase capabilities and may be termed **GDP**-4-**keto**-6-**deoxy**-D-**mannose**-3,5-epimerase-4-reductase. Analysis on a molecular sieve column using fast protein liquid chromatography established a molecular weight of 63,100 for the native enzyme, whereas sodium dodecyl sulfate-polyacrylamide gel electrophoresis estabolished a subunit molecular weight of 31,500.
IT Miscellaneous Descriptors
GDP-L-**FUCOSE** GDP-D-MANNOSE NADH
RN 58-68-4 (NADH)
2438-80-4 (L FUCOSE)
3123-67-9 (GDP-D-MANNOSE)
15839-70-0 (**GDP**-L-**FUCOSE**)